

Susceptibility to Experimental Allergic Encephalomyelitis and
Cell-Mediated Immunity to Myelin Basic Proteins and Peptides
in Inbred Fischer Rats

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Summary

Inbred Fischer rats were challenged for experimental allergic encephalomyelitis (EAE) by the injection of guinea pig encephalitogenic protein (EP) in Freund's complete adjuvant. This was followed by weight loss and histological lesions of EAE in most rats, but no neurological symptoms were observed. In vitro cell-mediated immune response to sensitizing EP was demonstrated, as was a partial cross-reactivity to bovine EP. Four peptides of the guinea pig EP also gave a clear-cut in vitro stimulation, but not so strong as did the intact EP.



Introduction

Experimental allergic encephalomyelitis (EAE) is a widely studied animal model for the human demyelinating diseases. It is readily produced by the injection of the basic protein of myelin (also called encephalitogenic protein or EP) emulsified in Freund's complete adjuvant (FCA). In susceptible rats, various clinical criteria are used for EAE identification, the main developing symptoms being weight loss, flaccid tail, weakness, paralysis (hind leg), incontinence, and death. These symptoms, or at least the neurological ones, are generally agreed to be accompanied by histopathological changes with vascular cuffs of mononuclear cell infiltration in the central nervous system (CNS) and sometimes aseptic meningitis. Histological signs can occur without clinical signs. However, there has been some controversy whether clinical signs are always accompanied by histological changes. Levine et al. (1975) stated that "there is no basis for the supposition that autoimmunity can cause major neurological signs in the absence of inflammatory lesions in the nervous system".

Recently, Allegretti & Marušić (1976) reported the absence of cellular infiltration in 37 T-cell deficient WVM rats, challenged for EAE, although total paralysis was seen in 20 (13 died). In the control group, total paralysis was seen in 6/16 (2 died); in this group, cellular infiltration was registered (frequency not reported).

The findings by Lindh (1977c) also indicated that there might be a dissociation between these parameters of EAE; it was apparent in the backcross generation of F₁ (Lewis x PVG) with Lewis rats.

McFarlin et al. (1975a,b) reported that susceptible Lewis rats also mounted a cell-mediated in vitro response to the sensitizing EP, whereas resistant BN rats failed to do so. This was interpreted as the presence, in Lewis rats, of an Ir-EAE-gene, designated by Williams & Moore (1973), that controls a T-cell response against EP, whereas BN rats lack this gene.

Vandenbark & Hinrichs (1974) showed that immunization of Lewis rats with guinea pig or bovine EP gave similar in vitro cell-mediated responses to sensitizing EP (migration inhibition technique), although guinea pig EP is much more encephalitogenic. No significant cross-reactivity between the two proteins could be shown.

Spitler et al. (1972, 1975) studied guinea pigs immunized with bovine EP and found a dissociation between disease development and cellular immunity.

Lindh (1977a,b) examined Lewis and PVG rats and their F₁ (Lewis x PVG) hybrids and confirmed the well-known high susceptibility to EAE in the Lewis strain. The PVG strain was found to be relatively resistant, and the F₁ hybrids were intermediate. None the less, irrespective of genotype, all rats mounted the same degree of cell-mediated response in vitro to sensitizing EP (guinea pig or bovine in origin and thus with different

encephalitogenic activity). Furthermore, guinea pig EP sensitization caused a partial cross-reactivity to bovine EP and the reciprocal cross-reactivity also seemed to exist. The present paper extends our studies to a third strain, the Fischer rat, which has the same AgB type as the Lewis strain (Gasser et al. 1975).

Material and Methods

Rats. Inbred rats of the Fischer strain of both sexes were obtained from Møllegaards Avlscentrum, Ejby, Denmark. Food and water was provided ad lib.

Antigen. Guinea pig and bovine EP was prepared according to previously-described principles (Bergstrand 1971). Peptide derivatives (regions 1-42, 43-88, 89-169, and HNB-89-169, the latter prepared by chemical blocking of the tryptophan residue [residue 115] of peptide 89-169 with 2-hydroxy-5-nitrobenzylation) (numbering according to the revised amino acid sequence of bovine EP; see Brostoff et al. 1974) were prepared and characterized according to previously-described principles (Bergstrand 1971, 1973). All antigenic materials were supplied by Dr. H. Bergstrand.

Immunization. Each rat was immunized with an emulsion: 0.05ml in each hind leg paw. The emulsion was composed of equal parts of FCA (Difco) and i) a saline solution of the guinea pig EP (50µg/rat) or ii) only saline (for controls). Each rat received either 125µg (FCA 5X) or 250µg (FCA 10X) of Mycobacterium butyricum. The mycobacterial content of FCA was thus concentrated five or ten times by centrifugation.

Experimental plan. 23 rats were divided into four groups and immunized with guinea pig EP and FCA 5X on different occasions with freshly prepared emulsions. Five rats were injected with guinea pig EP and FCA 10X, five with saline and FCA 5X, and five with saline and FCA 10X. The age was 3-4 months (5 months for saline injected rats) and initial weight between 150 and 400g. The rats were weighed daily and observed for possible neurological symptoms. After 28 days, the regional (popliteal fossa) lymph nodes were excised bilaterally and the CNS was removed for histological study.

Histological preparation and scoring. This was performed according to Lindh (1977a) and is only briefly summarized. Seven predetermined levels of the CNS were scored blind, and the sum of these gave a "histological score". If no lesion of EAE was found at a certain level, the score was 0; scores 1-3 described increasing degrees of histopathological findings with respect to both strength and abundance.

Lymphocyte cultures. For preparation of cultures, see Lindh (1977b). Thus, to the lymph node cells (LNC) cultures, either saline, PPD (purified protein derivative of tuberculin)

(Statens Seruminstitut, Copenhagen, Denmark), bovine EP, or guinea pig EP (of antigens: 50 μ g/tube) were added. The lymphocytes from five of the rats, injected with guinea pig EP and FCA 5X, were also tested with one of the following peptides of the guinea pig EP: sequence 1-42, 43-88, 89-169, and HNB-89-169, all tested in amounts equimolar to that of the guinea pig EP. After a culture period of three days, 3 H-methyl-thymidine was added for one hour, and radioactivity was assayed as described previously (Lindh 1977b). The counts registered during two 10-minute periods was logarithmated ($\log_{10}$), and this value was used in calculations. The "effect" value at a certain antigenic treatment is thus the difference between the mean $\log_{10}$ c.p. 20min. value in antigen-treated cultures and that in saline-treated cultures (controls) and represents the $\log_{10}$ stimulation index.

Results

Clinical signs. No neurological signs were found in any of the examined 38 Fischer rats. Table 1 presents the results and also gives some corresponding values for the rats of other genotypes, previously presented (Lindh 1977a).

The incidence of weight loss more than or equal to 10% of weight after a period of normal weight gain was 8/23 and 2/5, resp. among rats injected with guinea pig EP in FCA 5X and FCA 10X, resp. Incidence of weight loss less than 10% of weight after a period of normal weight gain and extending for at least three days was 9/23 and 3/5, resp. None of the 10 rats injected with saline and FCA displayed any similar weight loss.

Histological signs. Fischer rats injected with guinea pig EP in FCA 5X and FCA 10X, resp. displayed histological signs of EAE with an incidence of 15/23 and 3/5, resp. The medians in these two groups were 2 and 3, resp. (table 1). The incidence of animals without any sign of EAE was 4/23 and 0/5, resp. (not in table). No histological signs of EAE were found in any of the 10 rats injected with saline and FCA.

Figure 1 shows the correlation between mean histological score and clinical "degree" of EAE in rats injected with guinea pig EP and FCA 5X. There is some overlapping in the individual histological score. On the whole, weight loss less than 10% tends to be accompanied by a higher score than among "healthy" rats, and rats with weight loss more than 10% tend to display the highest histological score. In comparison with the Lewis, PVG, and F₁(Lewis x PVG) rats combined (Lindh 1977a), there seems to be a lower histological score in the Fischer rats for a given "degree" of EAE. For "degree" III, this difference is statistically significant ($p < 0.05$, Wilcoxon's rank test).

In vitro cell-mediated immunity to PPD, bovine EP, and guinea pig EP. Figure 2 shows the "effect" values ("stimulation index") recorded in the in vitro LNC stimulations with PPD, bovine EP, and guinea pig EP in rats injected with guinea pig EP and FCA 5X.

Table 1. Summary of signs of EAE in rats of different genetic background. Data from present paper and Lindh (1977a).

"Genotype"	Injected with	Incidence of neurological signs			Incidence of weight loss		Incidence of histological signs		Median for histological cal score
		31/36	≥10%	<10%	31/36	5/36	34/36	10/11	
Lewis	guinea pig EP + FCA 5X	31/36	31/36	5/36	34/36				12
F ₁ (Lewis x PVG)	" " + "	1/11	4/11	4/11	10/11				6.5
Fischer	" " + "	0/23	8/23	9/23	15/23				2
PVG	" " + "	0/10	0/10	0/10	10/10				2
Fischer	" " + " 10X	0/5	2/5	3/5	3/5				3
"	saline + " 5X	0/5	0/5	0/5	0/5				0
"	" + " 10X	0/5	0/5	0/5	0/5				0

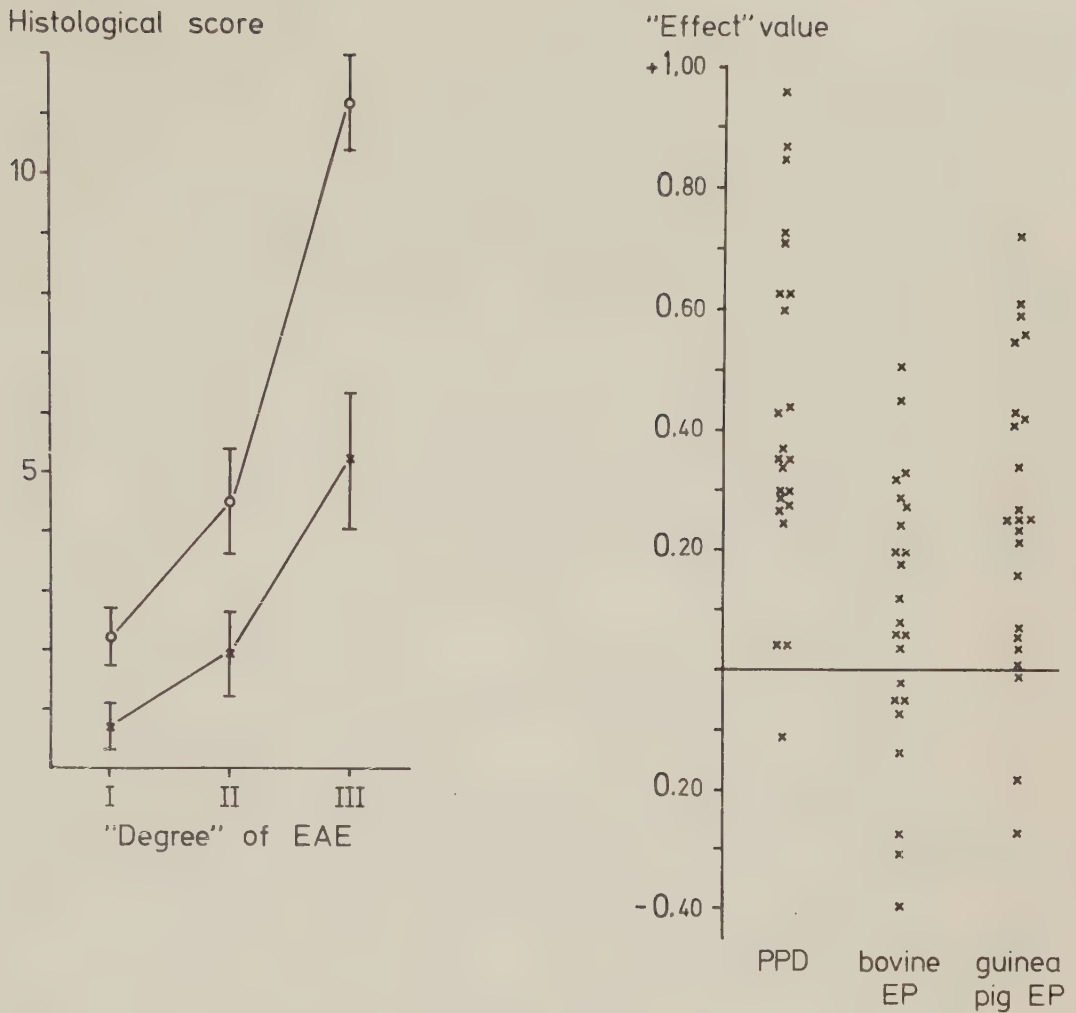


Figure 1. (left) The relation between mean histological score and clinical signs of EAE is illustrated for the 23 Fischer rats immunized with guinea pig EP in FCA 5X (crosses) with standard errors of the means given. Corresponding means for similarly treated Lewis, PVG, and F₁(Lewis x PVG) rats combined are also given (circles), data from Lindh (1977a). I = no clinical signs of EAE; II = weight loss less than 10% of the weight at the beginning of the loss and extending for at least three days; III = weight loss more than 10%.

Figure 2. (right) LNC from 23 Fischer rats, injected 28 days earlier with guinea pig EP in FCA 5X, were stimulated with PPD, bovine EP, or guinea pig EP. The "effect"-values are calculated as differences between uptake of ³H-methyl-thymidine (log₁₀c.p.20min.) with antigen added and with saline added. Each cross represents the mean of four tubes (one rat).

PPD gave the strongest stimulation in vitro with a mean "effect"-value of 0.43 ± 0.058 ($t = 7.5$, $p < 0.001$). Three of the rats showed no definite effect with PPD. Also guinea pig EP

gave a significant stimulation with a mean effect value of 0.26 ± 0.054 ($t = 4.8$, $p < 0.001$). Although a marked stimulation was seen in some animals, most showed only a weak response and seven none whatever. The stimulation seen with bovine EP was even weaker: a few animals gave a significant stimulation, but the mean effect value was only 0.09 ± 0.049 ($t = 1.9$, $0.05 < p < 0.1$). Figure 3 presents the actual recorded $\log_{10}$ c.p. 20 min. values in one experiment with significant effects with all three antigens. As previously shown, injection with FCA and saline will cause no in vitro cell-mediated response to guinea pig or bovine EP (Lindh 1977b).

In order to obtain an estimate of the cross-reactivity between bovine and guinea pig EP, the method described by Lindh (1977b) was used. This multiple regression studies the relation between the three variates: X_1 = control (saline) uptake, X_2 = "effect"-value for guinea pig EP (difference between uptake in culture with guinea pig EP added and culture with saline added), and Y = uptake obtained with bovine EP:

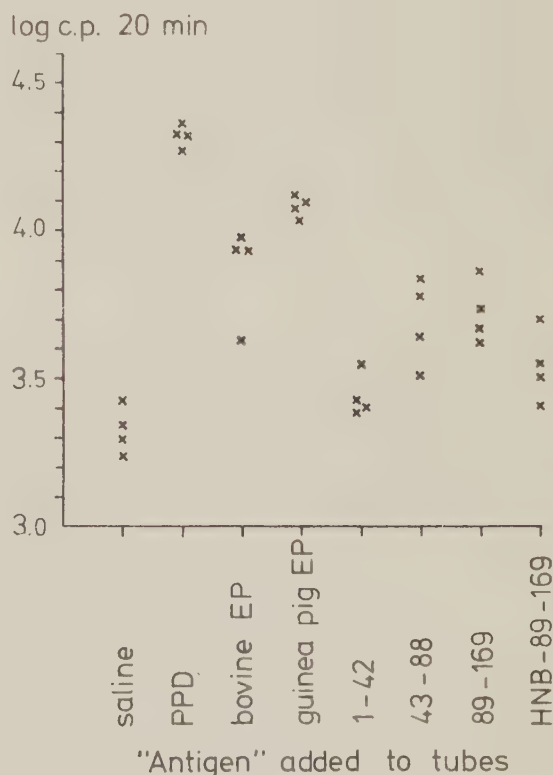
$$\hat{Y} = a + k_1 \times X_1 + k_2 \times X_2$$

a , k_1 and k_2 are constants. The equation obtained on this material was:

$$\hat{Y} = 1.47 + 0.56X_1 + 0.40X_2$$

The $\hat{Y}$ value is thus practically equal to half the control (saline) uptake + a constant, 1.47, + 40% of the "effect" obtained with guinea pig EP. The k_2 coefficient, 0.40, differs significantly from 0 ($p < 0.001$) and from 1 ($p < 0.001$).

Figure 3. Recorded $\log_{10}$ c.p. 20 min. values in one experiment (one rat) with significant effects with PPD, bovine EP, and guinea pig EP. Minor effects were recorded with the peptides. Each cross represents one tube.



Stimulative ability of peptides derived from guinea pig EP. In an effort to localize the sites in the guinea pig EP responsible for the stimulation in vitro, four peptides derived from guinea pig EP were tested on LNC from five of the Fischer rats used above for LNC stimulations. Figure 3 presents the recorded $\log_{10}$ c.p.20min. values in one experiment with peptides added to cultures. Peptides were tested in concentrations equimolar with that used for the guinea pig EP, and the results were treated according to the method used by Bergstrand & Källén (1973a,b,c) and expressed as per cent values of the total "effect" of the guinea pig EP. Table 2 summarizes the results. Stimulative capacity was obtained with all four peptides, with a possible preponderance for peptide 43-88. Blocking of the tryptophan site with HNB tended to slightly reduce the stimulative ability of peptide 89-169, but this could be random. No clear-cut "master site" with the main capacity to stimulate LNC to proliferation was thus demonstrable.

Discussion

Most of the Fischer rats injected with guinea pig EP and FCA showed some sign of EAE (weight loss or histological lesions). No neurological signs of EAE were found, although this was previously reported in this strain under similar, but not identical, conditions by Levine & Sowinski (1976). Possibly, an "augmentation" of the immunization procedure, such as increasing the amount of mycobacterium content or addition of pertussis vaccine, could have precipitated neurological signs (Levine & Wenk 1963, 1965, Levine & Sowinski 1976) but this was not seen in the present study. However one must recognize that the Fischer rats used in this study represent a substrain other than that used by Levine & co-workers cited above and could be more resistant to EAE. Gasser et al. (1975) reported the incidence of 5/5 of histological signs of EAE in Fischer rats injected with guinea pig spinal cord in FCA, but did not report on clinical signs. Probably, the amount of mycobacterium

Table 2. Stimulatory "effect" of peptides derived from guinea pig EP expressed as per cent of the "effect" of the intact EP. Calculations performed according to principles described by Bergstrand & Källén (1973a,b,c). Differences against 0 and 100% are tested with t-tests. LNC were taken from five Fischer rats immunized with the guinea pig EP in FCA 5X 28 days earlier.

Peptide	"degree" of reactivity (%)	differs from	
		0%	100%
1-42	44 $\pm$ 15	p < 0.02	p < 0.02
43-88	64 $\pm$ 15	p < 0.02	NS
89-169	56 $\pm$ 15	p < 0.02	p < 0.05
HNB-89-169	41 $\pm$ 15	p < 0.05	p < 0.02

that each rat received in that study was comparable with that in our study.

The data obtained in the present study on Fischer rats can be compared with those previously presented (Lindh 1977a) using identical experimental conditions but Lewis or PVG rats or their F_1 (Lewis x PVG) hybrids (table 1). The Fischer strain is definitely more resistant than the Lewis strain; no neurological signs develop in the former. Compared with the PVG strain, both absence of neurological signs and degree and incidence of histological changes are very similar, but the Fischer strain showed more weight loss. From this aspect, the Fischer rats resembled very closely the F_1 (Lewis x PVG) hybrids. From all available criteria, the Fischer strain thus appears to be intermediate between the Lewis and the PVG strains.

It can be noted that the Fischer strain - at the same degree of clinical signs, i.e. weight loss - shows a somewhat lower histological score than that found in the previous study for Lewis, PVG, and F_1 (Lewis x PVG) rats (comparing rats injected with guinea pig EP and FCA 5X). If weight loss is considered to be a valid criterion for EAE, this could indicate a dissociation between the two EAE criteria - weight loss and histological changes - of a nature similar to that previously discussed by the author (1977c) on neurological signs and histological changes. Weight loss is obviously not a consequence of the FCA injection as none of the 10 control rats displayed this symptom.

In vitro cell-mediated response. Lindh (1977b) reported that the Lewis, PVG, and F_1 (Lewis x PVG) rats did not differ in their cell-mediated in vitro responses to bovine and guinea pig EPs, although the same material showed differences between strains concerning the susceptibility to EAE. An analysis of variances, concerning the three variates: uptake in 1) saline (control) culture, 2) culture with bovine EP added, and 3) culture with guinea pig EP added, will give $F = 1.19$ (NS), for testing between genotypes Lewis, PVG, F_1 (Lewis x PVG), and Fischer. That study also reported that immunization with guinea pig EP would cause a cross-reactivity to bovine EP of approx. 50%. The recorded corresponding value for the Fischer rats is very close to this, 40%. The cross-reactivity probably reflects the similarities in the amino acid sequences between these two EPs. The present data thus support those we published previously: that the degree of stimulation in vitro appears very similar in all rats irrespective of genetic background and susceptibility to EAE. As mentioned in the Introduction, the literature shows that this is not true for the more resistant BN rat strain, where no in vitro cell-mediated reactivity to EP has been reported.

The major encephalitogenic site for rats is believed to lie within the amino acid residue sequence 43-88 (Bergstrand 1977). The present study, similar to that published previously (Lindh 1977b), demonstrated that a marked in vitro reactivity is seen also with peptides derived from regions outside sequence 43-88. This should be seen in the light of the weak encephalitogenicity that has been ascribed to peptides 1-42 and 89-169 (Martenson

et al. 1975). The in vitro reactivity to these peptides might reflect: 1) weak encephalitogenic sites, or sites cross-reacting to the major encephalitogenic site within peptide 43-88, 2) non-encephalitogenic sites, or 3) both these alternatives. In the present paper, the peptide 43-88 showed the strongest reactivity, not significantly different from that seen with the entire protein; but in the study using Lewis rats, no such difference between the different tested peptides was seen. The responses seen with LNC from Lewis rats were stronger than those recorded in the present study, but this difference might be random. Probably, all tested peptides are able to stimulate the cells from both rat strains in a similar way. There are thus no indications that the difference in EAE susceptibility between the Lewis and the Fischer strain parallels a differential in vitro sensitivity to the major encephalitogenic site residing within the 43-88 sequence.

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